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Neocarus spelaion sp. n. (Parasitiformes, Opilioacaridae), a new species of cave dwelling *Neocarus* from Minas Gerais state, Brazil

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Abstract

A new cave dwelling species of the genus *Neocarus* (Parasitiformes: Opilioacarida: Opilioacaridae) is described from adult females and males collected in Minas Gerais state, southeast Brazil. Five or six *d*-type setae on palps, females with pregenital area hairless, genital setae smooth, cylindrical ovipositor with two terminal lobes, and males with genital and pregenital setae variable in shape, some being smooth and pointed, others ribbed and pointed, are the combinations of characters that define the new species.

Keywords

Opilioacarida, Hypogean, Neotropical

Introduction

The order Opilioacarida presently includes 13 genera, 47 described species and one sub-species. Of these, only four genera, but more than half of all species and the described subspecies are from the New World. (Juvara-Bals and Baltac 1977, Vázquez and Klompen 2002, 2009, Bernardi Silva et al. 2013, Vázquez et al. 2015). Most species are found in forest litter, but some have been recorded in hypogean environments,

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for example *Caribeacarus brasiliensis* Bernardi, Silva, Zacarias, Klompen & Ferreira, 2013, *Caribeacarus vanderhammeni* (Juvara-Bals & Baltac, 1977), *Neocarus caipora* Bernardi, Klompen & Ferreira, 2014, *Neocarus coronatus* Araújo & Feres, 2018, *Neocarus orghidani* Juvara-Bals & Baltac, 1977, *Neocarus potiguar* Bernardi, Zacarias & Ferreira, 2012, and *Neocarus proteus* Bernardi, Klompen, Zacarias & Ferreira, 2013. Recently, a large number of species, including a new genus, have been found and described from Brazil (Araújo et al. 2018, Bernardi et al. 2012, 2014, Bernardi et al. 2013, Vázquez et al. 2014, 2015). In addition to these new taxa, in the present paper were describe a new species, *Neocarus spelaion* sp. n., from caves in Minas Gerais state, southeast Brazil.

Material and methods

Study area

The species of opilioacarid described in this present study was observed in caves located in karst areas in Minas Gerais state, Brazil (Fig. 1). All specimens were found at the entrance to caves, among soil litter or under rocks. Caves where this species is found are part of the Cerrado biome (Brazilian savanna) and are located south of the Bambuí Geomorphological unit ("Grupo Calcário Bambuí"), a large karstic formation encompassing four Brazilian states (Minas Gerais, Bahia, Goiás and Tocantins). Unfortunately, collections were not conducted in adjacent epigean environments, but it is believed that this species is not restricted to caves and could be distributed in a much larger area than that studied, as with other species of Brazilian Opilioacarida found in caves and karst areas (Bernardi et al. 2012, Bernardi et al. 2013a, b).

All specimens were collected by hand from caves, searching under stones, in accumulations of organic matter, cracks in the soil and were stored in vials with 70% ethanol. Posteriorly, all specimens were prepared as slide-mounted specimens. For this purpose, specimens were dissected (due to size), cleared in Nesbitt's solution, and mounted on slides using Hoyer's medium (Walter and Krantz 2009).

Drawings were prepared using a Zeiss Axioscope 3 phase contrast microscope, connected to a drawing tube; and measurements were taken in the same scope and are presented in micrometers (μm). All measurements were taken from slide mounted specimens. Ultrastructural analyses were also conducted through use of scanning electron microscopy. A female was placed on an aluminium support stub covered with a film of aluminium foil with carbon tape, sputter-covered with gold (Baltec SCD 050), and observed in a LO EVO 40 XVP scanning electron microscope. The nomenclature of setae and other morphological characters follows Grandjean (1936), van der Hammen (1969, 1976, 1977), Vázquez and Klompen (2002, 2009), Bernardi et al. (2014), and Klompen et al. (2015).

Specimens are deposited at the Mite Reference Collection, Department of Entomology and Acarology, Escola Superior de Agricultura "Luiz de Queiroz" (MZLQ), Universidade de São Paulo, Piracicaba, São Paulo, Brazil; Collection of Subterranean Invertebrates (ISLA), Section of Zoology, Department of Biology, Universidade Federal

Figure 1. Collection site of *Neocarus spelaion* sp. n. in the State of Minas Gerais, Brazil (star).

de Lavras, Lavras- MG, Brazil; Collection of the Acarology Laboratory (UFMG-AC), Department of Zoology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

Results

Arachnida Lamarck, 1802
Acari Leach, 1817
Parasitiformes Reuter, 1909
Opilioacaridae With, 1902
***Neocarus* Chamberlin & Mulaik, 1942**

***Neocarus spelaion* Bernardi, sp. n.**
http://zoobank.org/0FB7E13D-C2BD-42C9-99FA-C084474E1F2C

Material examined. Holotype 1 female specimen deposited at MZLQ, Brazil, Minas Gerais, Matozinhos, Cave 013, 19°31'57.21"S, 44°5'30.30"W Datum WGS84, col. Fi-

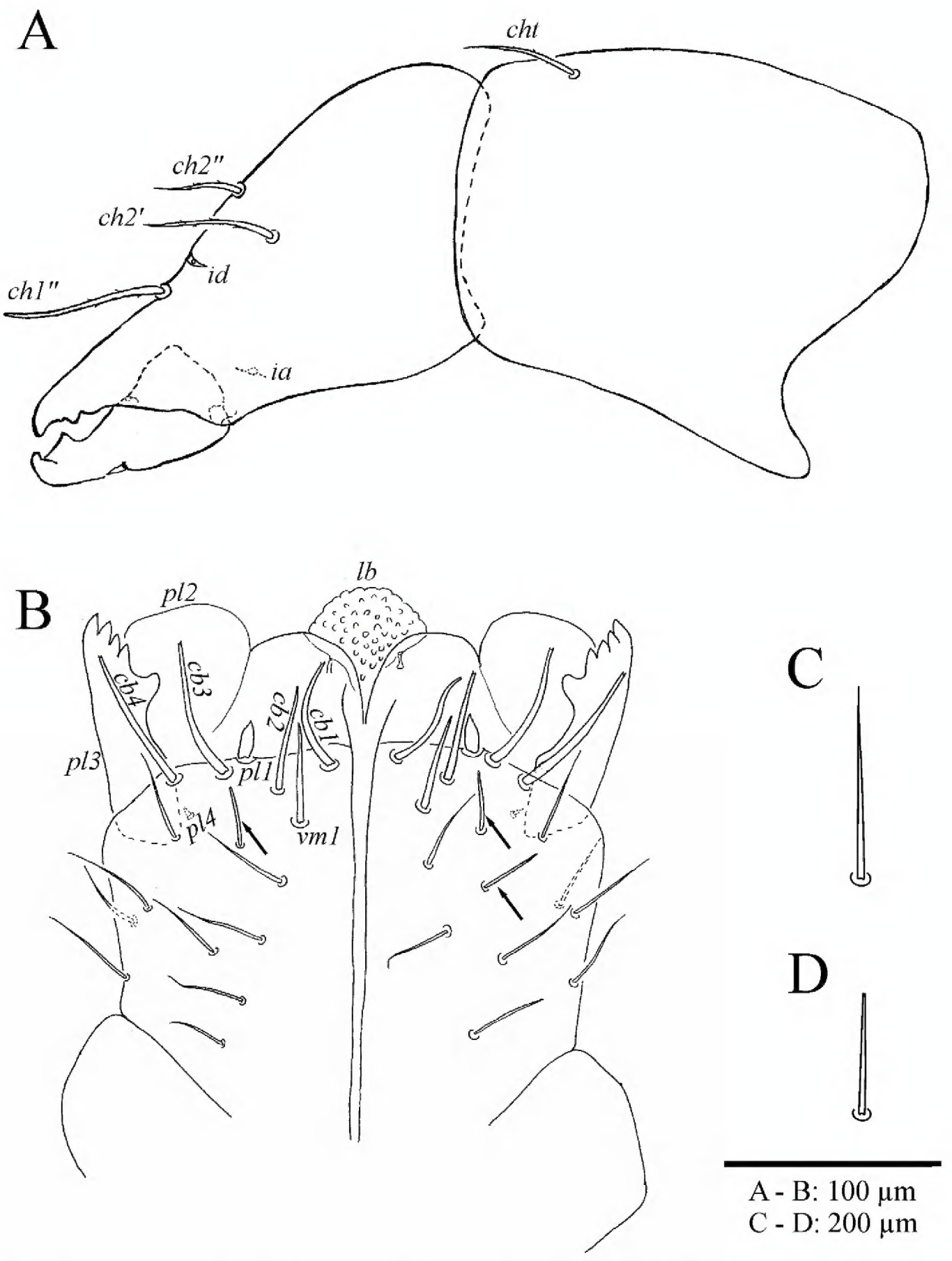

Figure 2. *Neocarus spelaion* sp. n. (Female): **A** Lateral view of chelicerae **B** Ventral view of subcapitulum **C** Detail of a seta with a fine tip **D** Detail of a seta with a rounded tip, found only in females (arrows in Fig. B). Abbreviations; *cb1–4* = circumbuccal setae, *pl1–4* = paralabial setae, *ch* = cheliceral setae, *cht* = basal segment seta, *lb* = labrum, *id* = dorsal lyrifissure, *iα* = antiaxial lyrifissure.

lho et al., setember.2016, manual collection. Paratype 1 male specimen deposited at MZLQ, same data as holotype; paratype 1 male specimen deposited at ISLA, same data as holotype; 1 female specimen deposited at ISLA, same data as holotype; para-

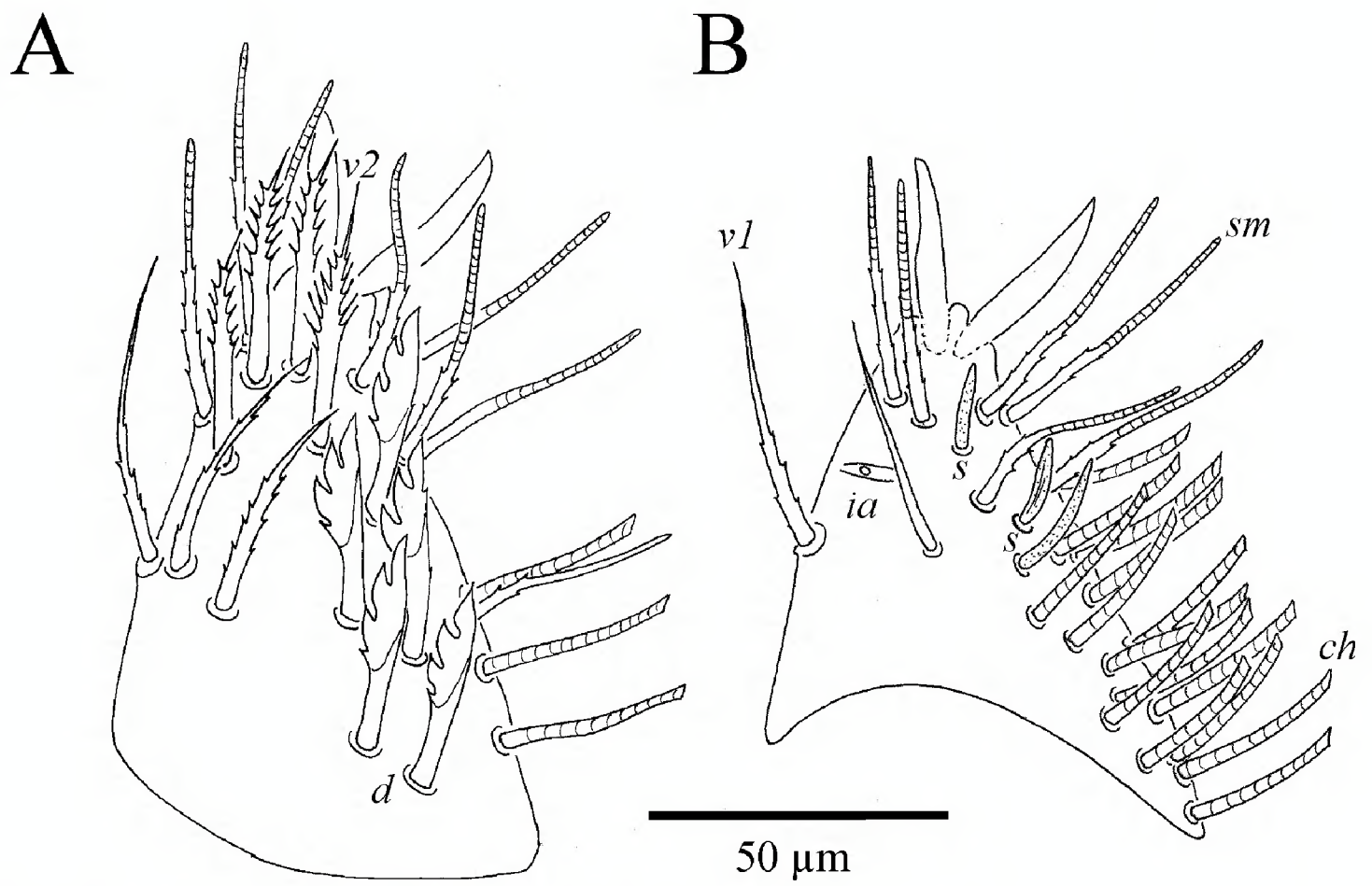

Figure 3. *Neocarus spelaion* sp. n.: Ventral (**A**) and dorsal (**B**) view of the palp tarsus.

types 1 male specimen deposited at MZLQ, Brazil, Minas Gerais, Matozinhos, Cave 053, 19°31'47.92"S, 44°5'33.51"W Datum WGS84, col. Filho et al., setember.2016, manual collection; 1 female specimen deposited at MZLQ, Brazil, Minas Gerais, Matozinhos, Cave 053, 19°31'47.92"S, 44°5'33.51"W Datum WGS84, col. Filho et al., setember.2016, manual collection; paratype 1 female specimen deposited at ISLA, Brazil, Minas Gerais, Matozinhos, Cave 093, 19°31'56.06"S, 44°5'34.70"W Datum WGS84, col. Filho et al., setember.2016, manual collection; paratype 1 female specimen deposited at UFMG-AC, Brazil, Minas Gerais, Matozinhos, Cave 010, 19°31'58.45"S, 44°5'31.05"W Datum WGS84, col. Filho et al., setember.2016, manual collection.

Etymology. Noun in apposition. The specific epithet "spelaion" is in honor of the Spelayon, a group of speleologists and for their contribution to the knowledge of Brazilian subterranean biology and geospeleology.

Diagnosis. *Neocarus* species are often identified by a combination of characters, mainly using palp, pregenital and genital setae, while the ovipositor shape is usually considered a species-specific character. A summary of the characters used for species definition is presented in Table 1.

The definition of the species *Neocarus spelaion* sp. n. is given by; palp tarsus with 5 or 6 foliate setae (*d*-type); area between sternal and genital verrucae with two pairs of long, tapering setae; female with hairless pregenital area and 10-12 smooth genital setae, and ovipositor cylindrical, bare and with two terminal lobes. Male genital area with 7–11 setae, and pregenital area with 9–12 setae variable in shape, some of them

Table 1. Comparative setal patterns for the pregenital and genital region of *Neocarus* adults.

Occurence	Species/Subspecies	Female		Male		Palp	
		Pregenital region	Genital region	Pregenital region	Genital region	*ch*-type	*d*-type
		No. and type of setae	No. and type of setae	No. and type of setae	No. and type of setae		
North America							
USA	*N. texanus*	2 st/r	0	4–6 st/r	8–9 sh	10–14(21[a])	5
Mexico	*N. nohbecanus*	nude	0	4–5 st/r	5–7st/r	17–19	4
Mexico	*N. siankaanensis*	nude	0	2 st/r	4 st/r	14–15	5
Mexico	*N. bajacalifornicus bajacalifornicus*	2 st/r	0	5–8(13[a]) st/r	7–8(11[a]) st/r	14–18 (21[a])	5
Mexico	*N. bajacalifornicus chamelaensis*	2–3 st/r	0	4–5 st/r	4–6 st/r	16	5
Mexico	*N. calakmulensis*	2–3 st/r	0	2–6 st/r	3–8 st/r	17	5
Mexico	*N. veracruzensis*	2 st/r	0	6–8 st/r, 0–1 s	6–8 st/r	13	5
Mexico	*N. comalensis*	5–7 st/r	3 st/r			14–18	5
Mexico	*N. chactemalensis*	nude	nude	4–6 st/r	4–6 st/r	11–13	4
Central America							
Nicaragua	*N. nicaraguensis*	2–5 st/r	0	2–7 st/r	3–6 st/r	18–22	5 or 6
Cuba	*N. orghidani*	nude	0	4–5 st/r	5–7 st/r	20–24	4
South America							
Venezuela	*N. ojastii*	nude	0	6–9 ?	13 st/r	–	–
Brazil	*N. proteus*	2–5 st/r	4–6 wb	2–5 st/r	3–5 sh	12 or 13	5 or 6
Brazil	*N. potiguar*	1 tp/r	4–8 sh	5 st/r	7–10 st/r	25–27	5 or 6
Brazil	*N. coronatus*	nude	6–8 tp	1–7 st/r	5–15 tp/r	18–25	4
Brasil	*N. spelaion*	nude	10–12 sh	9–12 sh or tp/r	7–11 sh or tp/r	14–18	5 or 6
Brazil/Argentina/ Uruguay	*N. platensis*	0–2 st/r	6–9 sh	6–10 st/r	5–10 sh	14	5 or 6

[a] - number of setae in super adults; sh: smooth setae; st/r - stout and ribbed setae; tp - tapering; tp/r: tapering and ribbed setae; wb: weakly barbed setae.w

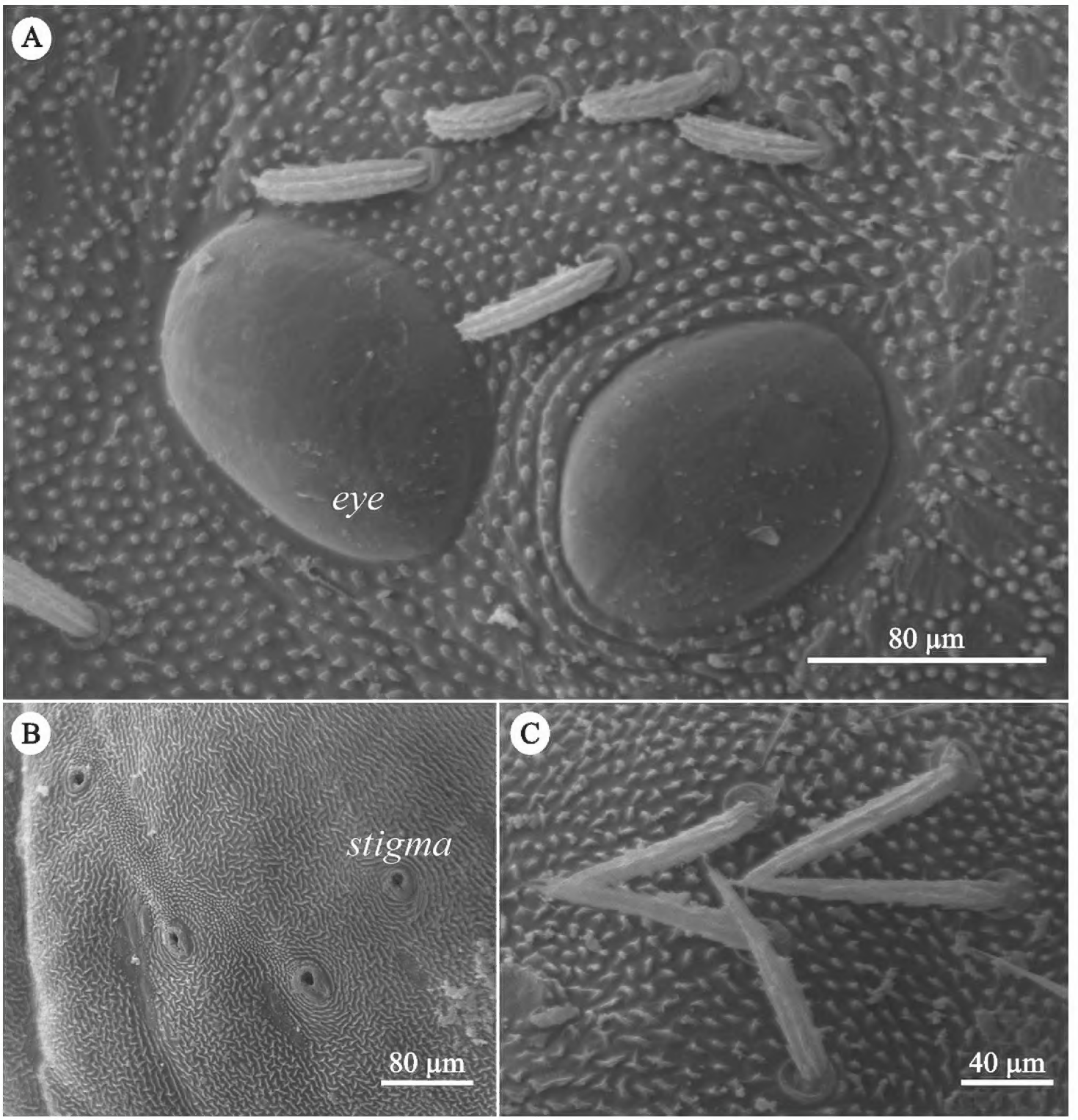

Figure 4. *Neocarus spelaion* sp. n. (female): **A** View of eyes and prodorsal setae **B** View of the stigmas **C** View of the sternitogenital setae (barbed and tapering).

smooth and pointed, or ribbed and pointed. Ovipositor with two terminal lobes and shape of setae on male pregenital and genital area are unique to *N. spelaion* sp. n.

Chelicera (Fig. 2A). Basal segment (170–212 µm) with 1 and fixed digit (237–267 µm) with 3 setae. Setae *ch1"*, *ch2"*, *cht* and *ch2'* lightly barbed in some specimens (only visible at high magnification). Dorsal (*id*) and antiaxial lyrifissure (*ia*) well developed. Fixed digit with 1 tooth, movable digit (80–87 µm) with 1 tooth and a well-developed terminal hook. Movable digit with one ventral denticles in the basal portion. Internal surface of movable digit with an orifice of cheliceral gland (*ocg*). Sexual differentiation indistinct or absent.

Subcapitulum (Fig. 2B). All 4 pairs of paralabial setae present: *pl1* small, conical; With's organ (*pl2*) membranous and barbed marginally; rutella (*pl3*) with one distinct

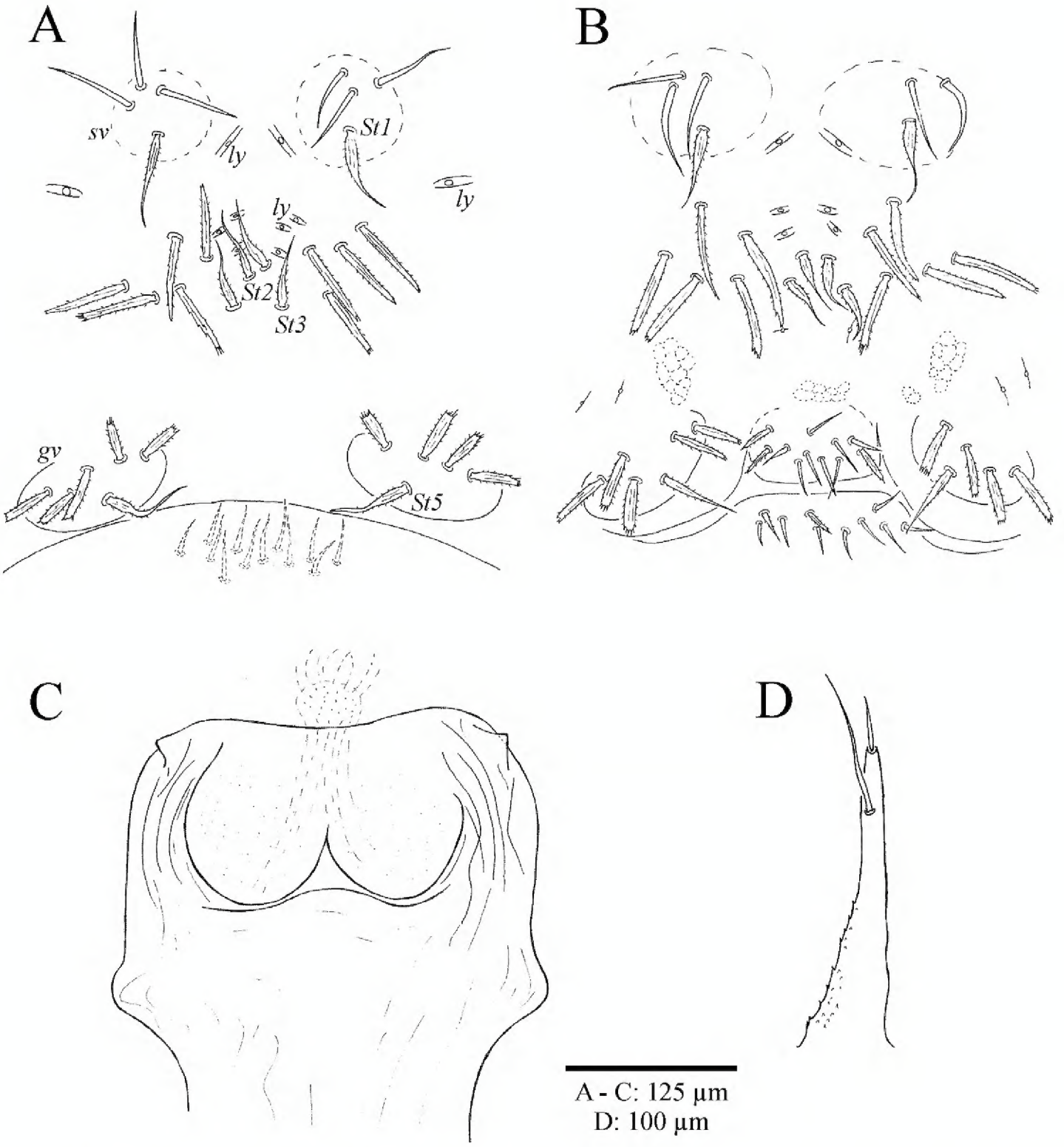

Figure 5. *Neocarus spelaion* sp. n.: **A** View of female sternitogenital region **B** View of male sternitogenital region **C** View of invaginated ovipositor **D** Sternapophyses. Abbreviations; *sv* = sternal verrucae, *gv* = genital verrucae, *st1–5*= sternitogenital setae, *ly* = lyrifissure.

row of 5 teeth, inserted dorso-lateral; *pl4* very small, inserted dorsal. With 4 circumbuccal (*cb*), and 8–9 median and subcapitular setae on male and 10–12 on female. Seta *vm1* in all adults with rounded tip and robust, similar to circumbuccal setae. Females with one to three additional pairs of median setae with rounded tips (indicated by arrows in Fig. 2B, C). All setae in the median area of the subcapitulum in males show a fine, attenuated tip. Canals (*ogl1* and *ogl2*) on lateral lips distinct.

Palp tarsus (Fig. 3A–B). Trochanter with 2–4 *r* and 2 *p* setae; Femur with 4–9 *p*, 6–16 *r* setae. Spike-like setae absent. Genu with 1–7 *p*, 16–29 r setae. Tibia with

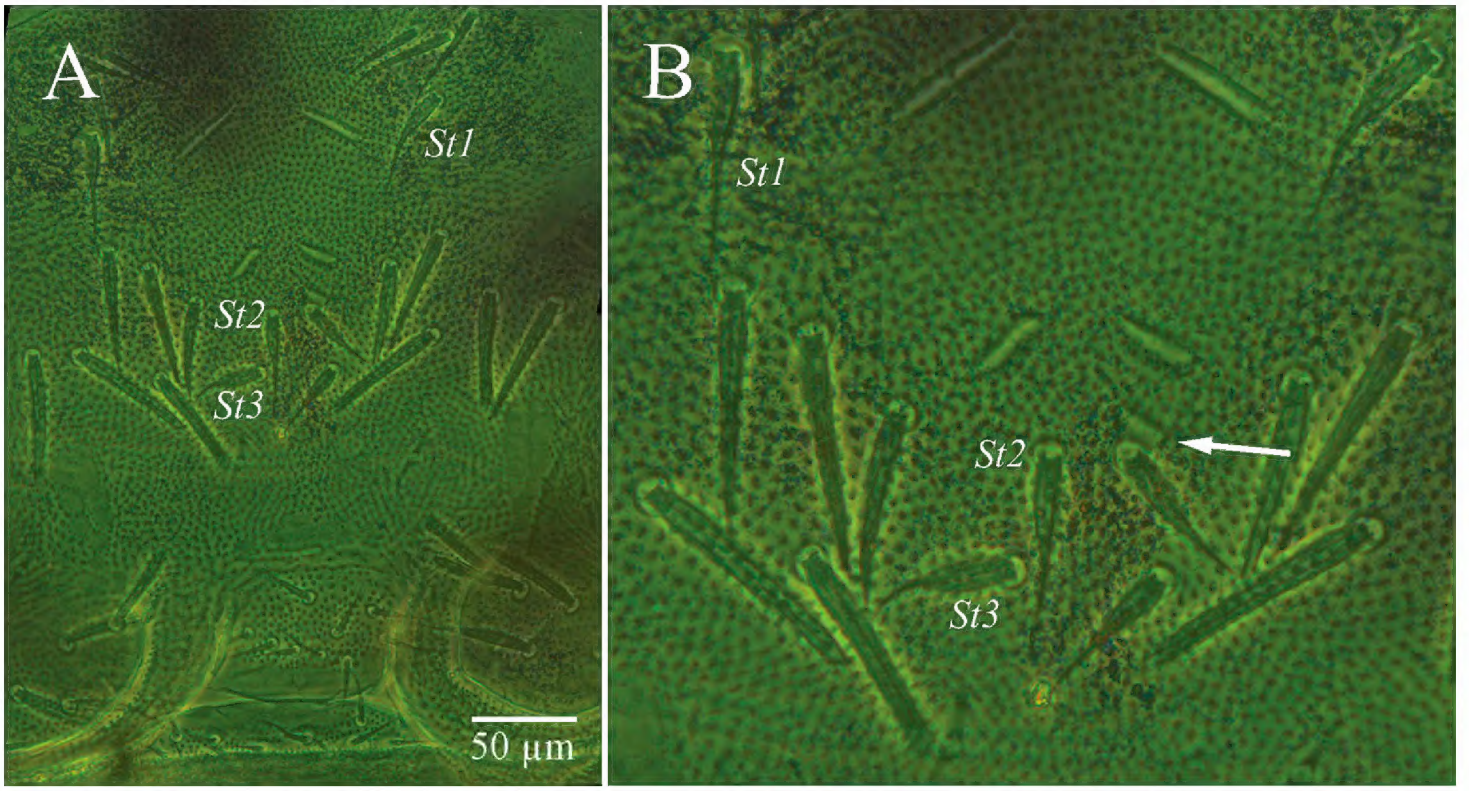

Figure 6. *Neocarus spelaion* sp. n.: **A** View of male sternitogenital region **B** Details of the duplicated lyrifissures. Abbreviations; *St1–5* = sternitogenital setae.

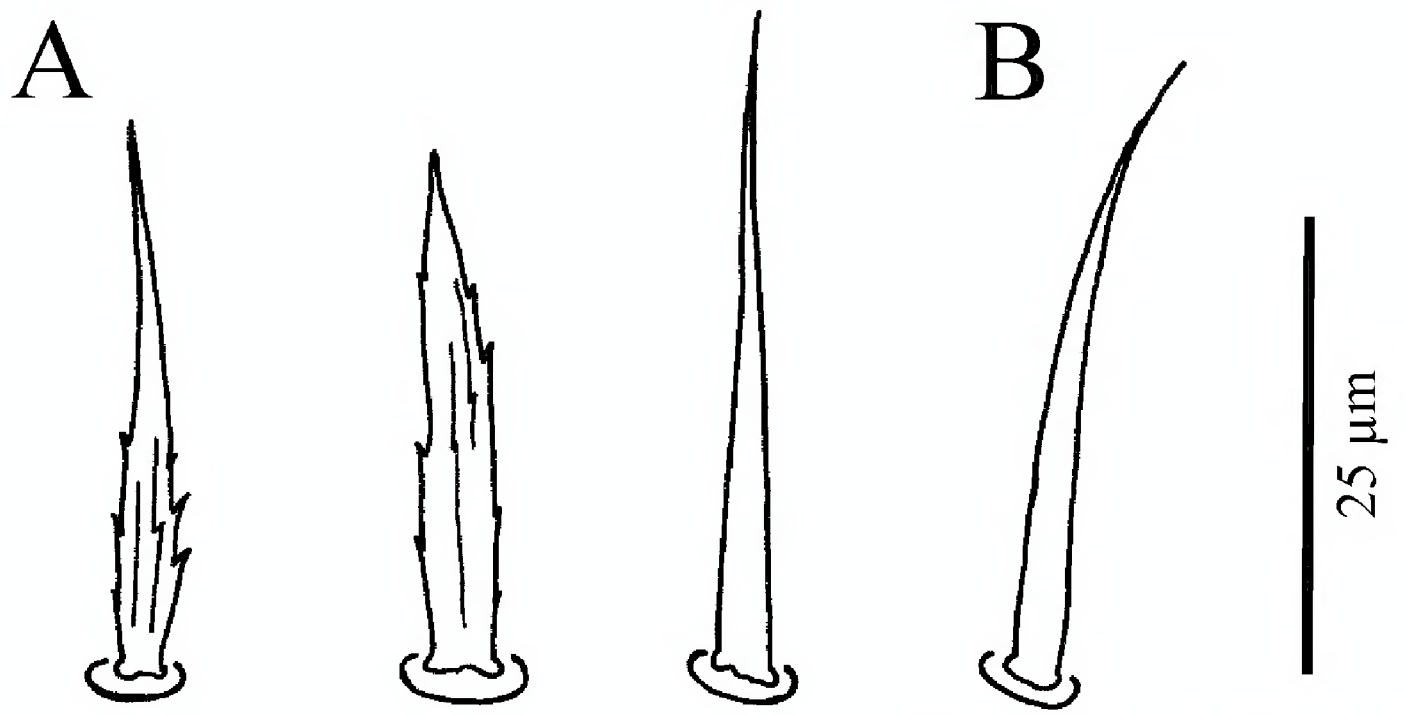

Figure 7. *Neocarus spelaion* sp. n.: **A** Details of variation in morphology found in pregenital and genital setae in male **B** Detail of female genital setae.

16–30 *s* (some of these setae have small and fell barbs on base, and are positioned dorso-laterally), 16–27 *r*. Palp tarsus with lyrifissures *iα* and *iπ*. Setation includes two or one pointed and smooth setae positioned dorsally (probably *v1*), three *s*-type (smooth, resemble solenidia, but lack the transverse striation), 5–6 *d*-type (leaf-like), 3–4 *v1*-type, 3–5 *v2*-type, 14–18 *ch*-type and 7–8 *sm*-type setae. Pretarsus with well-developed claws. Total length of palp (trochanter/femur/genu/tibia/tarsus) 205–240 mm. No distinct sexual differentiation observed.

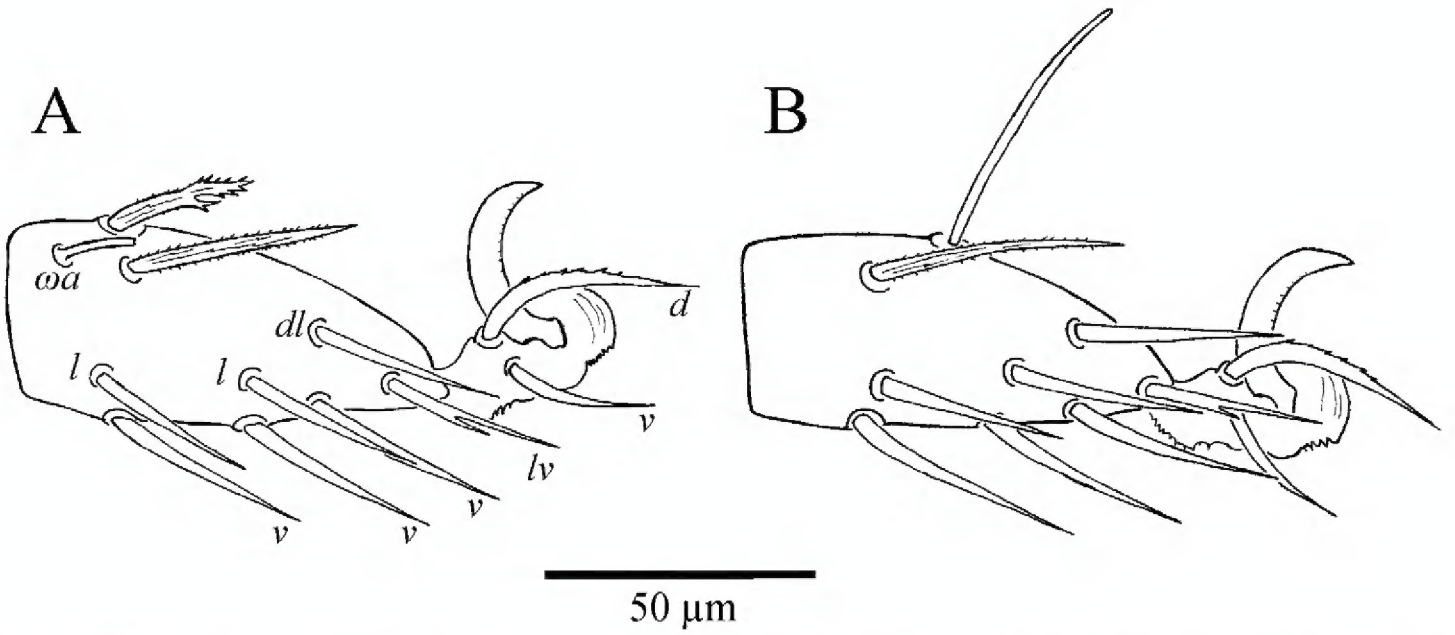

Figure 8. *Neocarus spelaion* sp. n.: Acrotarsus II, female; **A** posterolateral view **B** anterolateral view. Abbreviations; ω*a* = solenidia, *d* = dorsal setae, *l* = lateral setae, *dl* = dorsolateral setae, *v* = ventral setae, *lv* =lateroventral setae.

Idiosoma (Fig. 4A–B). Length 2100–2200; width 11600–12400, no significant diferences were observed among male and female**.** Color light with dark blue patches. Body sometimes with a brownish background resulting from ingested food. Dorsal shield with 130–172 ribbed setae and two pairs of eyes. One pair of lyrifissures present (same position and shape as observed by Klompen et al. 2015). Rostrum rounded. Dorsal portion of idiosoma between the shield and the preanal segment without setae, but with numerous lyrifissures arranged in transverse rows. Four stigma is present in separated body segments (VIII, IX, X and XI). Preanal segment with 1 dorsal and 2 lateroventral setae; anal plates in adults each with 10–12 stout, ribbed setae. Sexual differentiation indistinct or absent

Sternitogenital region (Figs 5–7). Sternapophyses with two setae, one small seta at the tip and one long and barbed seta positioned more basal. Sternal verrucae on adults with 2–5 barbed, tapering setae plus 1distinctly longer, barbed, tapering setae (*St1*). Remaining sternal area with 2 pairs of barbed, tapering setae (*St2* and *St3*) on central area, and 4-6 pairs of long, ribbed setae, and commonly 3 pairs of lyrifissures, two pairs very large, the third smaller; all different in shape and size from "standard" opisthosomal lyrifissures. But some specimens have the central pair of lyrufissure duplicated (placed between *St2* and *St3*) (Fig. 6A, B). Setae *St2* and *St3* equal in size. Pregenital capsules each with 1 long tapering seta (*St*5) and 4–5 ribbed setae. Pregenital and genital area in male with setae variable in shape, some of them smooth and pointed, or ribbed and pointed. Male genital area with 7–11 setae, and pregenital with 9–12 setae. In females, pregenital area nude and genital area with 10–12 smooth and pointed setae. The genital setae of the new species present a clear base but are hidden when the ovipositor is folded inside the body of the specimens. This group of the setae is considered genital and not eugenital, because eugenital setae are considered all setae present near or at tip of the ovipositor, without a clear base, like described by Vázquez and Klompen (2009).

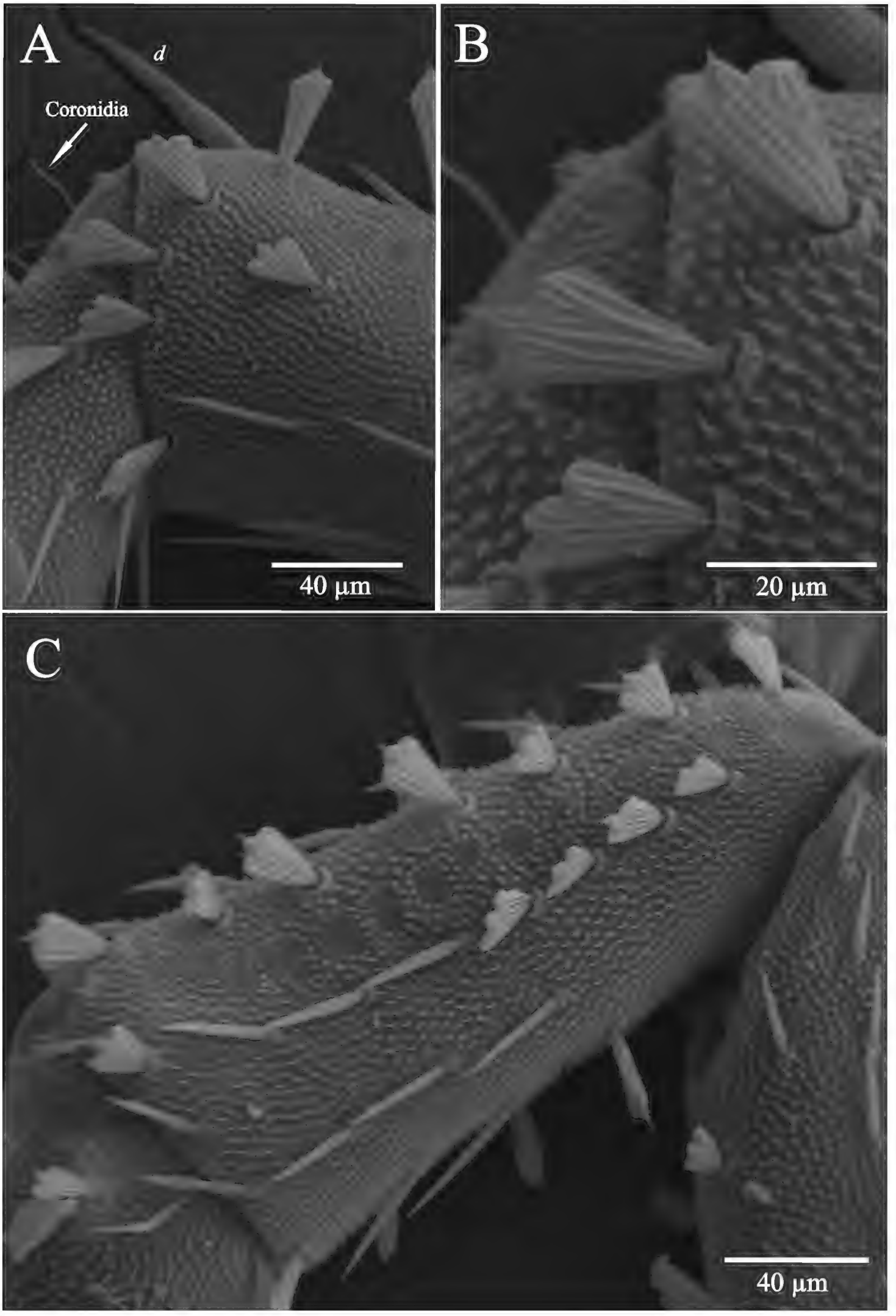

Figure 9. *Neocarus spelaion* sp. n.: **A** Lateral view of basitarsus and tibia, arrow indicating coronidia and mucronate setae (*d*) **B** Detail of the papiliform setae on tibia II; B lateral view of genu II.

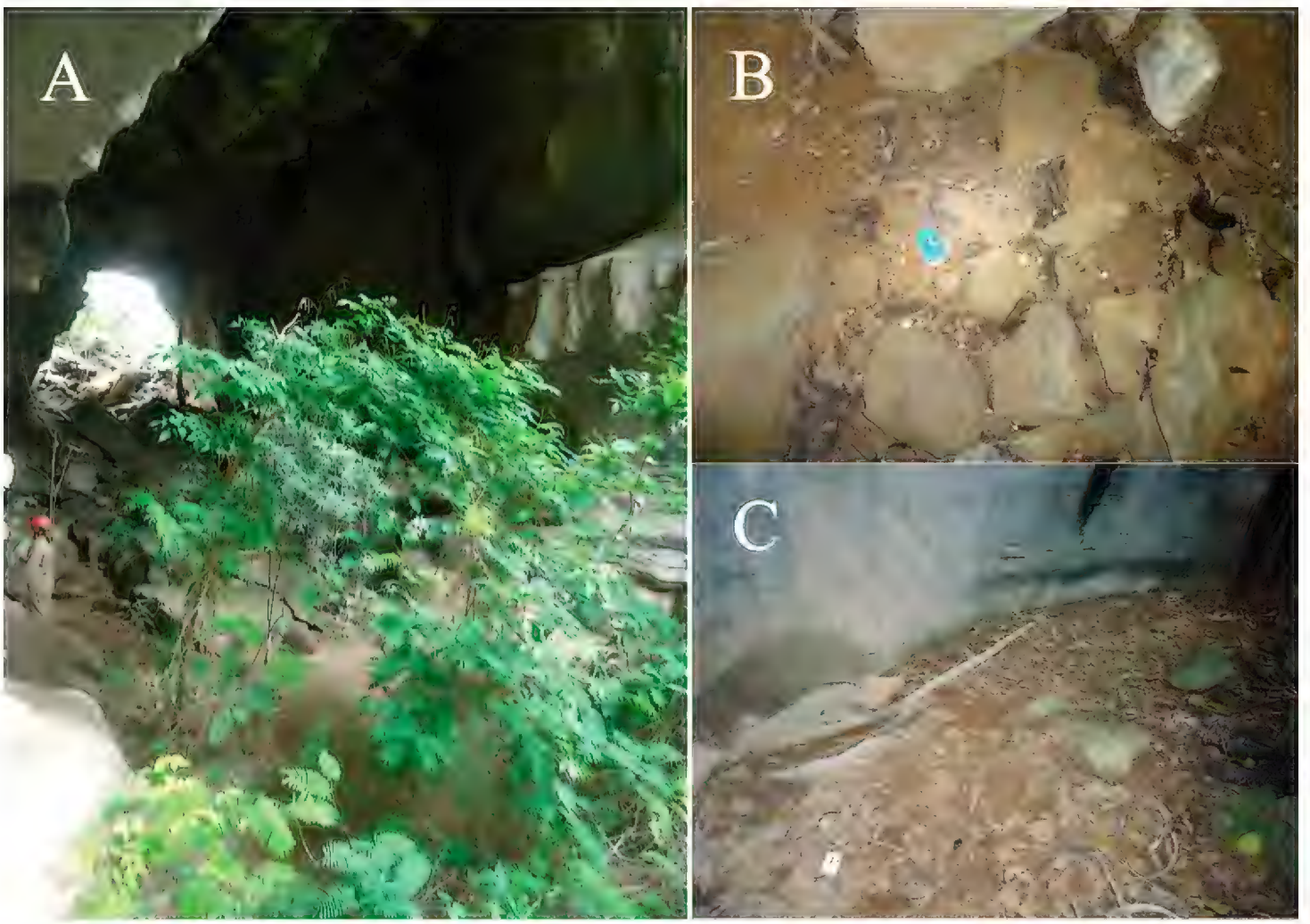

Figure 10. General aspects of the places where the specimens of *Neocarus spelaion* sp. n. were found: **A** overview of the cave **B, C** Substrates where the specimens were found.

Ovipositor when invaginated, consisting of a tube-like structure with two rounded and lateral lobs on tip, but when evagined these two lobs turn in a single structure, placed laterally to opening, without a eugenital setae. A single pair of gland-like structures in its median portion, and apex convex.

Legs (Figs 8–9). Leg I longer than others (leg I, 4310–4360 μm; leg II, 2135–2340 μm; leg III 2185–2375 μm; leg IV, 3295–3725 μm). Telotarsus I has a highly modified group of dorsal setae located in the apical portion, close to the tarsal claws, homologous to the Haller's organ of ticks (Moraza 2005). All other leg segments carry three types of setae arranged in distal to basal lines: 1) tapering and barbed, 2) papilliform and 3) smooth seta.

Acrotarsus of leg II with a dorsal bifurcate seta and two smooth sensilla (one long and other ω*a* small) resembling solenidia (Figure 7). Acrotarsi of legs III–IV carry just 3 long, barbed and tapering setae dorsally. Additionally, acrotarsi II–IV carry 3 pairs of smooth ventral setae, 1 pair of lightly barbed ventrolateral setae (positioned distally), 1 pairs of smooth lateral setae and 1 pair of smooth laterodorsal setae (positioned distally). Basitarsi II–IV carrying coronidia dorsaly and two solenidia, ω*d* dorsodistal and partially sunk, and ω*p* basidorsal. Pretarsi in all instars with one pair of claws and 2 pairs of setae, one pair long and curved, and other smaller and apically pectinate. Pretarsal ambulacrum rounded and smooth.

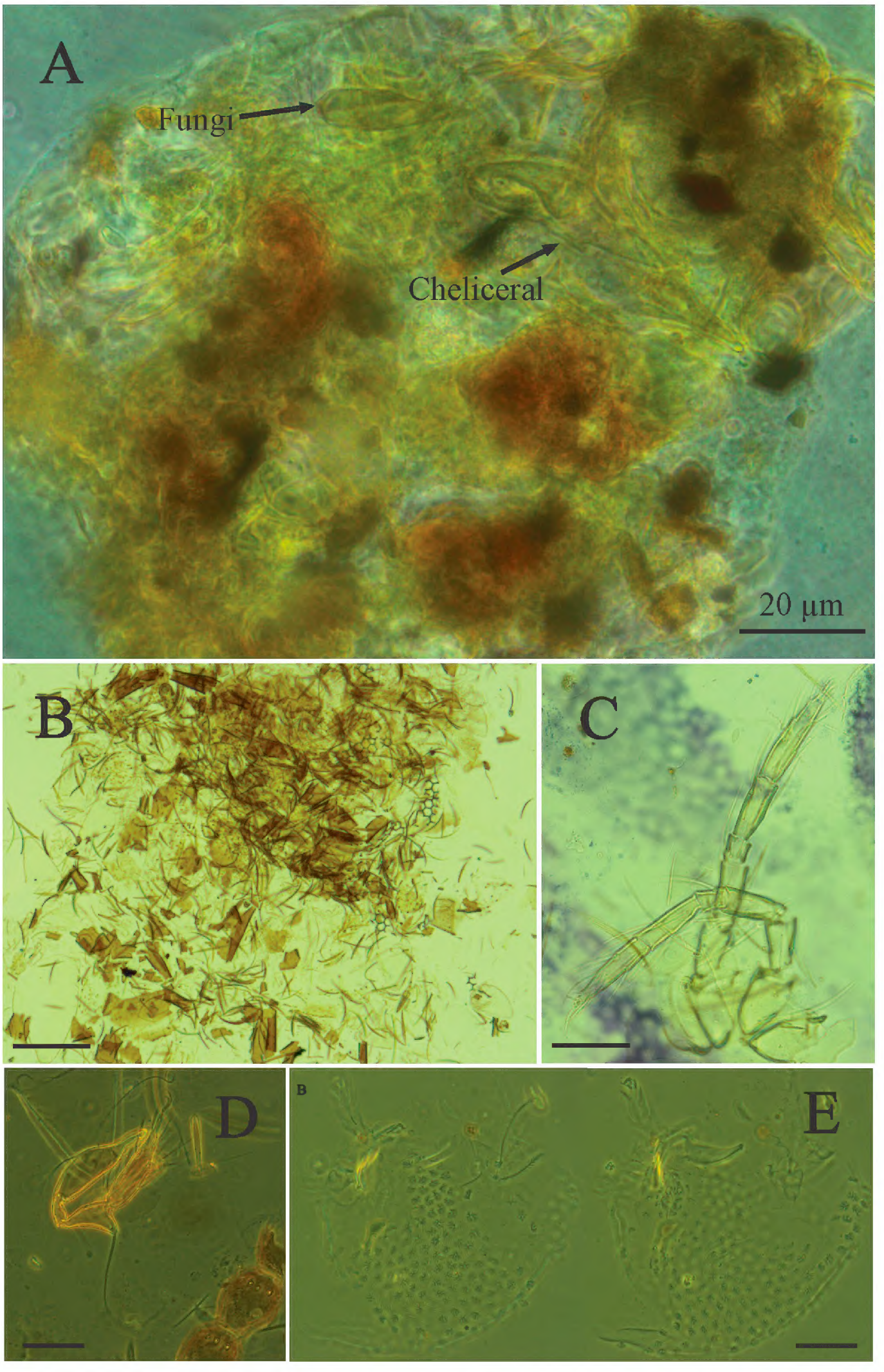

Figure 11. General aspects of the gut content of the *Neoacrus spelaion* sp. n.: **A** overview of the pellet with indigested food **B** plant tissue fragments and **C** mite part (Parasitengonina larvae) **D** Oribatida leg **E** Alycidae specimen.

Remarks. The caves where the *N. spelaion* sp. n. was found receive large amounts of organic matter from the surrounding epigean system, which penetrates through skylights, gaps, cracks in the ceiling and at the main entrance. These caves contain a lot of loose rock, plus wide entrances, resulting in extensive photic zone in the hypogean environment, which enables the development of plant species in places near the entrance, providing a suitable place for shelter and permanence of Opilioacarida species (Fig. 10). However, it is believed that this species is not restricted to the cave environment and can be distributed in a much larger area than that studied, as found for other species of Brazilian Opilioacarida that inhabit caves and karst areas (Bernardi et al. 2012, Bernardi et al. 2013ab).

The transport of organic matter from the epigean to the hypogean environment can be an important factor for the opilioacarid species to establish a population in the interior of caves. There have already been reports that this species group feeds on pollen, fungi hyphae, plant fragments and arthropods (With 1904, van der Hammen 1966, Vázquez and Palacios-Vargas 1989, Walter and Proctor 1998, Klompen 2000). Analyses of the stomach contents of *N. spelaion* sp. n. specimens corroborated with these previous reports, revealing individuals had ingested plant tissue fragments, pollen, fungal spores, besides exuviae from invertebrates and mites (parts of Mesostigmata, Oribatida, Parasitengonina larvae and Alycidae specimen) (Fig. 11). The intact exuviae of mites (Acariformes: Oribatida) suggested that these individuals had been swallowed whole. As noted by Walter and Proctor (1998), fragments or even nearly complete bodies of other mites (*Speleorchestes*, Eupodidae, Teneriffiidae, Tarsonemidae and Oribatida) can be found in the fecal masses of Opilioacarida, but species in this group have never been observed preying on other organisms. Thus, it is likely that they scavenge the remains of dead mites and other invertebrates rather than actively predate these groups.

The Brazilian opilioacarids are widely distributed in karst regions, places where there is a great demand for mineral extraction. At least other 5 species, including a new genus, have been found in this formation, however, their descriptions have not yet been published (pers. observation).

The region of the Bambuí Geomorphological unit contains important limestone deposits and is under for intense pressure from mining activities, threatening caves and the surrounding epigean habitats (Ferreira et al. 2009, Simões et al. 2014). These activities threaten all species, due to the rapid and destructive nature of resource extraction methods, which often occur in an uncontrolled manner and which falls below the legally accepted standards.

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sobre novas espécies troglóbias de ácaros identificadas no Estado de Minas Gerais a partir dos exemplares depositados na Coleção Taxonômica da UFMG (CT–UFMG); number: 04/2016"). LFOB scholarships were provided by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). Thanks also to the reviewers (Mark Harvey and Marce Santos Araújo) for their valuable suggestions.

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